Transmission Electron Microscopy (TEM)

(JEOL JEM-1220, JEOL JEM-3010, JEOL JEM-ARM200CF)

Transmission Electron Microscopy (TEM) is used to look at the internal structure of a specimen. The specimen, which can be no larger than 3mm in diameter and 0.25mm thick in order to fit in the specimen holder, has to be further thinned to allow electrons to pass through parts of the specimen. Typically transmission thin areas must be less than 100nm thick, depending on the density of the specimen and the accelerating voltage of the microscope, and in general the thinner the specimen the better the images. For high resolution imaging thicknesses must be no greater than 50nm.

The wave-like nature of electrons was discovered in the early 20th Century and in 1924 Louis de Broglie proposed that their wavelength (λ) is given by:-

$$\lambda = h/(mv)$$

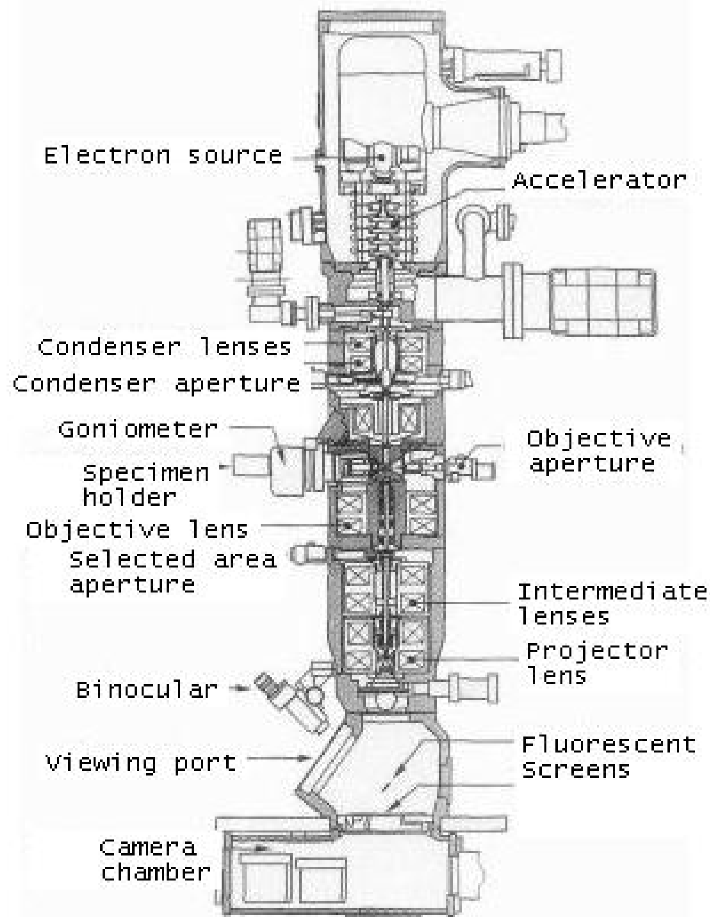
where h is Planck’s constant (= 6.626 x 10-14 Js), m is the mass of the electron and v is the speed. Raising the accelerating voltage above 50kV causes the electrons to speed up and the wavelength to shrink below 5pm. The higher energy electrons generated can penetrate several microns into a solid. In 1927 G.P.Thomson showed that if the solid was a thin crystalline specimen a transmission electron diffraction pattern could be obtained, exactly as in the case of x-rays. It was soon realized that, as negatively charged particles, the electrons could be focused using electric or magnetic fields and in 1931 Ruska had built the first Transmission Electron Microscope with two lenses. By 1933, after adding a third lens, Ruska was able to demonstrate resolutions somewhat better than that of the light optical microscope. The first commercial TEM, built by Siemens in 1938, had a resolution of 10nm at an accelerating voltage of 80kV. Such was the interest Metropolitan Vickers (UK – later AEI), RCA (USA) and Hitachi (Japan) were all building commercial instruments by 1941.

Ernst Ruska's three lens TEM in the Deutsches Museum Munich >

Today, although companies in USA, Holland, UK, Germany, Japan, USSR and Czechoslovakia have at one time manufactured transmission electron microscopes, competition, and a relatively small market, has reduced the number to three main manufacturers; JEOL and Hitachi (Japan), Thermo-Fisher (Holland).

Transmission Electron Microscopy has proved invaluable for examining the internal structure of materials. For example, although theoreticians had predicted the presence of dislocations in crystalline metals to account for their deformation at much lower forces than calculated for a perfect crystalline array of atoms, it took a TEM to directly image the dislocations. Modern instruments have sufficient resolution to image individual atomic planes in crystalline solids. TEM has been equally as useful in the life sciences. Nearly all organelles and cell inclusions were either discovered or resolved in finer detail using TEM. Such descriptions have laid the foundation in understanding cell function and understanding how cell structure varies in normal, experimental and diseased states.

The modern TEM is capable of displaying magnified images of a specimen, typically in the x2,000 to x1,500,000 magnification range. It can also produce electron-diffraction patterns and if fitted with XEDS or EELS micro-chemical or electronic state information.



The electron optical system of a TEM consists of an electron source and several electron lenses stacked vertically to form a lens column. The TEM can be conveniently divided into three sections:-

1) The ***illumination system*** consists of the electron source, electron accelerator, together with two or more condenser lenses which, together with a condenser aperture, determines the diameter of the electron beam at the specimen and the intensity level in the TEM image. Typically there will be gun and condenser alignment coils to allow the optical center of the gun and the condenser system to be aligned on the optical axis of the objective lens and also a condenser stigmator to correct for the imperfections in the condenser lenses.

2) The ***objective lens and specimen stage*** are the heart of the instrument. The specimen (which is typically 3mm in diameter and less than 100nm thick in the region of interest) is mounted in the specimen stage within the strong magnetic field of the objective lens (~2T). The electron optical properties of the objective lens will define the ultimate resolution of the microscope. The specimen holder and goniometer allows specimens to be held stationary while imaging at atomic resolution while also allowing movement in up to 5 axes (X, Y, Z and tilt X, tilt Y), depending on specimen holder, and easy transfer into and out of the microscope vacuum system. There is an objective stigmator in the lower bore of the objective lens which corrects for the axial asymmetry of the pole piece and an objective aperture, in the back focal plane, which can increase contrast by defining which electrons form the image.

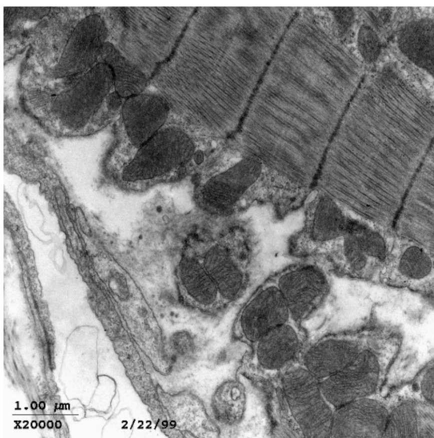
3) The ***imaging system*** consists of at least three lenses that together form a magnified image (or diffraction pattern) of the specimen on the fluorescent screen or CCD camera. Small changes to the intermediate lenses focal lengths allow the magnification to be changed in discrete steps over a large range (x2,000 – x1,500,000). Larger changes to the excitation of the first of these lenses (intermediate lens 1) are used to switch between imaging and diffraction on the viewing screen. In conjunction with the selected area aperture, an area of the specimen can be defined in imaging mode from which a diffraction pattern can be obtained in diffraction mode (selected area diffraction). The intermediate lenses are relatively weak with focal lengths of a few centimeters. Alignment coils in the imaging system allow fine movement of the image (image alignment) on the viewing screen and alignment of the imaging system with the center of the various cameras and detectors (projector alignment). The final lens (projector lens) is a strong lens (f = few mm) used to produce an image or diffraction pattern across the entire TEM viewing screen. A phosphor screen is used to convert the electron image to a visible form either as the viewing screen or the scintillator for a CCD camera. The traditional ZnS phosphor was chosen to give an image in the middle of the spectrum (yellow-green) to

which the eye is most sensitive. Alternative phosphors are available with better sensitivity and are used with CCD cameras.

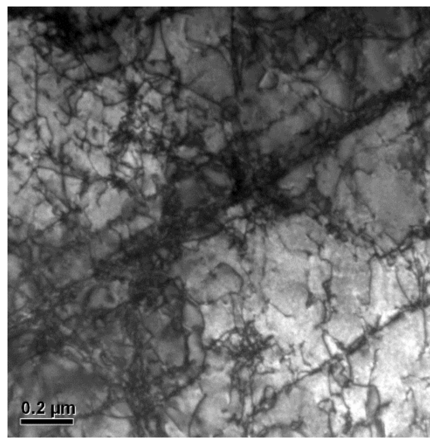
The majority of the column is kept at high vacuum. In the electron gun a sufficiently good vacuum is needed to prevent high-voltage arcing and also avoid oxidation of the electron emitting surfaces of the source. The required vacuum level will vary dependent on electron source from relatively poor vacuum for a tungsten thermionic source to ultra-high vacuum for a cold field emission source. In the column air is removed so that the electrons are not scattered by gas molecules. Typically the vacuum system of a TEM will be split into three zones separated by pneumatic valves. The ***Gun Vacuum Chamber*** contains the electron source and accelerator, the ***Electron Column*** all the lenses and specimen stage and the ***Camera (or Detector) chamber*** the fluorescent screen and CCD cameras.

In a transmitted light microscope, variations in intensity within an image is caused by differences in the *absorption* of photons in different regions of the specimen. In the TEM however, if the specimen is thin enough, nearly all incoming electrons are transmitted through the specimen. Some of these transmitted electrons are *scattered* by the specimen and this gives contrast in the final image. There are two main types of interaction. Those between incoming fast electrons and the atomic nucleus gives rise to elastic scattering where almost no energy is transferred, and those between incoming fast electrons and atomic electrons results in inelastic scattering where significant energy is transferred from the fast electron to the atomic electron. Both elastic and inelastic scattering will cause a change in direction of the fast electron.

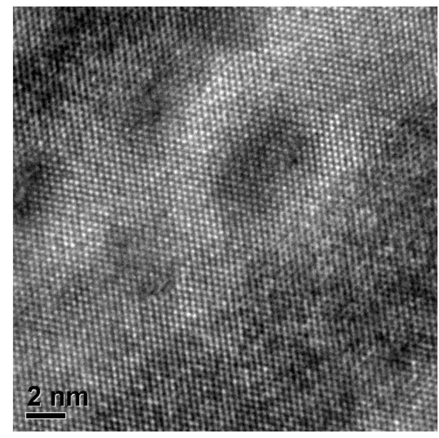
Most TEM images are collected with an objective aperture inserted around the optic axis of the microscope. If a small aperture is used, selecting only the direct beam, then any scattered electrons will fall outside this aperture and as a result the image will show contrast variations. If the specimen is amorphous this contrast will depend on the specimen thickness and density and a ***mass-thickness contrast*** image will be obtained. If the specimen is crystalline then any scattering contrast will be dominated by ***diffraction contrast*** caused by Bragg diffraction of electrons from suitably aligned lattice planes.



Mass-thickness contrast image of stained heart atrial muscle



Diffraction contrast image of disclocations in a steel

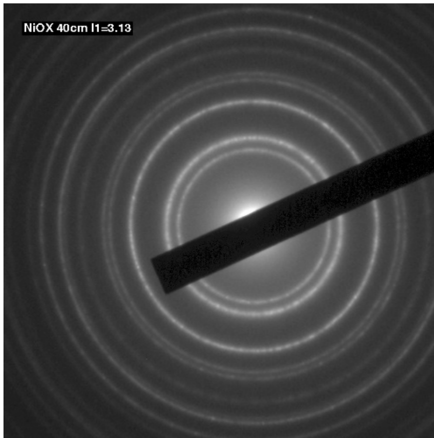


Phase contrast image of a silicon/germanium quantum well

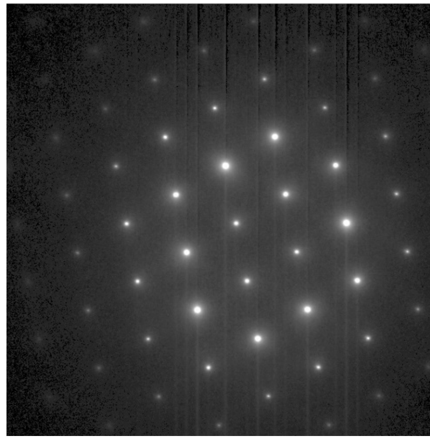
In addition to scattering contrast, features seen in some TEM images depend on the phase of the electron wave at the exit plane of the specimen. This cannot be measured directly in the TEM but does give rise to interference between electron beams that have passed through different parts of the specimen which can be bought together by defocussing the TEM image. A large diameter (or no) objective aperture is needed to enable many beams to contribute to the ***phase contrast*** image. In the

special case of a crystalline specimen oriented to be on a zone axis this can give rise to *atomic resolution* images, however it must be remembered that this is not a direct image of the structure. This can be seen if the defocus is changed from one side of focus to the other - the contrast will reverse with bright becoming dark and *vice versa*.

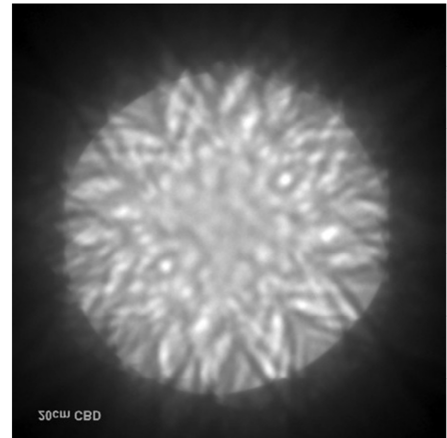
By changing the strength of the first intermediate (diffraction) lens below the specimen it is possible to focus the back focal plane of the objective lens, rather than the image plane, on the viewing screen and look at the diffraction pattern. Using the Selected Area Diffraction (SAD) aperture it is possible to choose a sub-micron area of the specimen to get diffraction patterns from. For smaller areas Convergent Beam Diffraction (CBD) can be used.



SAD pattern from a nano-crystalline Nickel Oxide specimen



011 SAD pattern from a single crystal Silicon specimen



011 CBD pattern from a single crystal Silicon specimen

Inelastic scattering also gives rise to other signals that are used for chemical and electronic characterization. In particular an incoming fast electron can cause ionization of an atom in the specimen by transferring energy to, and emitting an inner shell electron. The primary electron can continue down the column, having lost some energy, which can be detected using a magnetic sector spectrometer to disperse the electron beam as a function of energy (Electron Energy Loss Spectroscopy (EELS)). The ionized atom relaxes by an outer shell electron falling into the inner shell vacancy, which can lead to the emission of a characteristic X-ray. These X-rays can be collected with an Energy X-Ray Energy Dispersive Spectroscopy (XEDS). Both EELS and XEDS can give information on the chemistry of the specimen; EELS also gives information about the electronic structure of the specimen.